Crosslinked, glassy styrenic surfactants stabilize quantum dots against environmental extremes†‡

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Received 4th February 2009, Accepted 29th May 2009
First published as an Advance Article on the web 19th June 2009
DOI: 10.1039/b902275a

Semiconductor, quantum dot (QD) nanoparticles (including CdSe/ZnS, CdTe/ZnS, and CdSe) were encapsulated within crosslinked shells of amphiphilic polystyrene-*block*-poly(acrylic acid) block copolymer. Transmission electron microscopy revealed that each particle was surrounded by a uniform layer of copolymer, and that the average diameter of the resulting QD-core micelles was between 25 and 50 nm, depending on the conditions of particle assembly. Overall, we found that aqueous suspensions of these QDs were substantially more stable to heat and pH than particles with other surface preparations; we argue that the enhanced stability is due to the uniform, hydrophobic coating of polystyrene around each particle and the reinforcement of this layer by shell-crosslinking. The biocompatibility of these particles was investigated by microinjection of particle suspension into live zebrafish embryos. The particles permanently stained the fish vasculature, but did not interfere with the normal development of the fish. We propose that QDs encapsulated in crosslinked block-copolymer shells allow QDs to be used in biological or biotechnological protocols requiring harsh reaction conditions.

Water-soluble, semiconductor quantum dot (QD) nanoparticles have been recently adapted to a variety of biological protocols. They are particularly promising as dyes for analytical biochemistry and fluorescence microscopy, where their extraordinary photostability, narrow emission bandwidth, large two-photon cross-section, and long emission lifetime make them superior to organic fluorophores in many quantitation and imaging techniques. Several methods have been developed to solubilize QDs and to maintain their fluorescence in water, including surface functionalization by small-molecule,[1,2] polymer,[3–5] or biomolecule[6,7] ligands, as well as encapsulation within shells of amphiphilic random copolymer,[8–10] silica,[11–13] or lipid,[14] or within polymer beads.[15] Some of these coatings are incompatible with different physiological extremes, such as low or high pH or high temperature. Recently, Nie and coworkers published a comparative study on the stability and fluorescence intensity of QDs encapsulated within different acrylate copolymer structures,[16] and found that acrylate block copolymers were not as effective at stabilizing QDs against physiological extremes as acrylate graft copolymers. In an effort to further develop QD dyes that survive harsh physiological and biotechnological environments, we have encapsulated QDs within crosslinked poly(styrene-*block*-acrylic acid) (PS-*b*-PAA$_{XL}$) block-copolymer surfactants. We report that the fluorescence of the resulting particle-core, surfactant-shell structures is stable to extreme conditions (*e.g.*, $1 \leq$ pH ≤ 13, $T \leq 65$ °C) that can extinguish other ligand-, polymer- or surfactant-capped QDs. We also demonstrate that PS-*b*-PAA$_{XL}$-coated QDs permanently stain the vasculature of living zebrafish without losing fluorescence intensity under the shear of blood circulation.

PS-*b*-PAA-coated quantum dots were prepared by a method previously developed for encapsulating carbon nanotubes[17] and gold[18,19] and magnetic[20] nanoparticles, in which the copolymer surfactant is gradually desolvated to form micelles around the suspended particles. Here we report that this method is also successful for encapsulating TOPO-capped QDs (Scheme 1). In a typical procedure, PS$_{250}$-*b*-PAA$_{13}$ (M_n = 27,000 g/mol; PDI = 1.15)[18] and a toluene or THF solution of QD (CdSe, core-shell CdTe@CdS or core-shell CdSe@ZnS, Evident Technologies, Troy, NY) were first mixed in *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF), or a DMF/THF mixture. (See ESI‡ for experimental details.) Water was then added to this mixture *via* syringe pump at a constant rate, until the solution was >85% H_2O. The resulting suspension was dialyzed against pure water, exposed to 2,2′-(ethylenedioxy)bis(ethylamine) and EDC to crosslink the 25% of the carboxylates in the PAA block, and then extensively dialyzed again to remove excess reagents. The suspension was further purified by repeated centrifugation and redispersion to afford permanently stabilized, shell-crosslinked QD@PS-*b*-PAA$_{25\%XL}$ nanostructures.

Transmission electron micrograph (TEM) images of the nanoparticles show that a variety of QDs could be successfully encapsulated in PS-*b*-PAA (Fig. 1A–D). In all cases, the particles were confined near the center of the assembled polymer. The nanostructure of the product QD@PS-*b*-PAA$_{XL}$ depended on both the initial solvent composition and the rate of water addition. Slow water addition to QD-polymer mixtures containing some DMF resulted in singly encapsulated, fairly monodisperse nanoparticles (Fig. 1A), while faster water addition to particles and polymer in pure THF yielded micelles containing distributions of multiple particles (Fig. 1D). Regardless of the number of particles per micelle, the structures retained the spectral properties of the component QDs (Fig. 1E).

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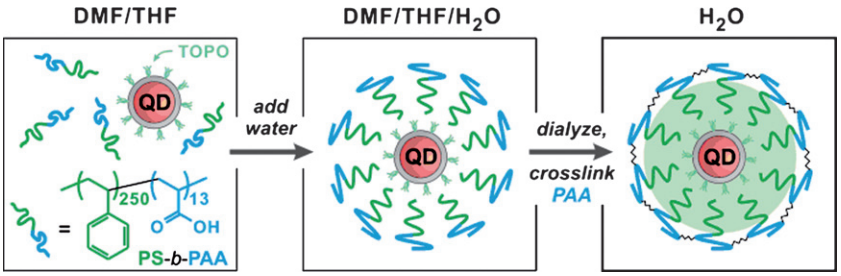
† This paper is part of a *Journal of Materials Chemistry* theme issue on inorganic nanoparticles for biological sensing, imaging, and therapeutics. Guest editor: Jinwoo Cheon.

‡ Electronic supplementary information (ESI) available: Detailed procedures of synthesis and characterization of QD@PS-*b*-PAA$_{XL}$, stability studies, and zebrafish injection, as well as additional TEM images. See DOI: 10.1039/b902275a/



Scheme 1 Encapsulation of QDs within block copolymer micelles.

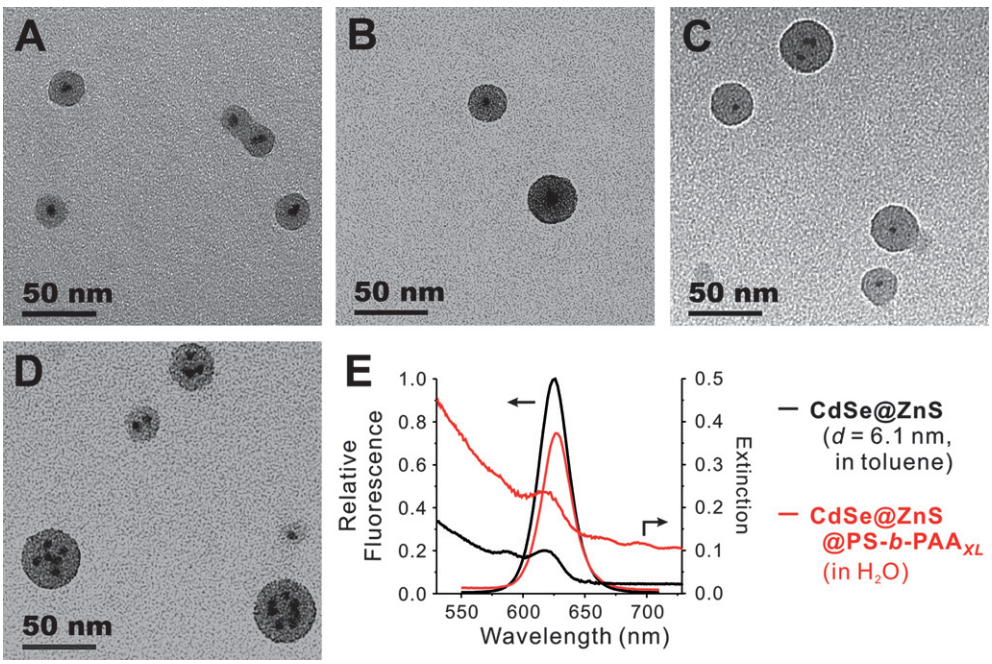


Fig. 1 Representative TEM images of encapsulated QDs within PS_{250}-*b*-PAA_{13} micelles: (A) 6.1 nm CdSe@ZnS, (B) 5.2 nm CdSe, (C) 4.3 nm CdTe@CdS, and (D) 6.1 nm CdSe@ZnS. Precursor particles were dissolved in a mixture of DMF/THF (20 vol% of DMF) for samples (A–C) and in pure THF for (D). (E) Extinction and emission spectra of QDs, before and after encapsulation in PS-*b*-PAA_{XL}.

Absorbance spectra after encapsulation show that the polymer shell contributed some scattering to the overall extinction of the suspension. In addition, the quantum yield of the encapsulated QDs was between 25% and 70% less than that of the TOPO-capped precursors, on a per-QD basis, depending on the QDs used. The excitation and emission maxima and the narrow emission range of the particles were unaffected by micelle encapsulation. These properties were unchanged over extended storage (over at least 1 month) in water or buffer solution. In general, suspensions of un-crosslinked particles were not as stable.

QD@PS-*b*-PAA_{XL} nanoparticles are more stable to extremes of pH, temperature, and competing ligands than other commercial, water-soluble QDs we tested. We compared suspensions of CdSe@ZnS@PS-*b*-PAA_{XL} (6.1 nm QD core, Fig. 1D) to three other carboxylate-functionalized, commercially available CdSe@ZnS particle types: QDs functionalized with α-thiol-ω-carboxyl-poly-(ethylene glycol) (HS-PEG-COOH) ligands (EviTag Type 1, Evident Technologies, Troy, NY), TOPO-capped QDs encapsulated by a carboxyPEGylated phosphatidylethanolamine (PE-PEG-COOH) surfactant[14] (EviTag Type 2, Evident), and TOPO-capped QDs surface-passivated with *n*-octylamine-modified PAA (P*n*OAm-*co*-PAA) copolymer[10] (Qdot 605 ITK, Quantum Dot Corporation, Hayward, CA). Overall, we found that CdSe@ZnS@PS-*b*-PAA_{XL} particles retained their fluorescence longer under extreme conditions than other surface-functionalized or surface-passivated QDs. For example, the fluorescence of block-copolymer-coated QDs at 65 °C

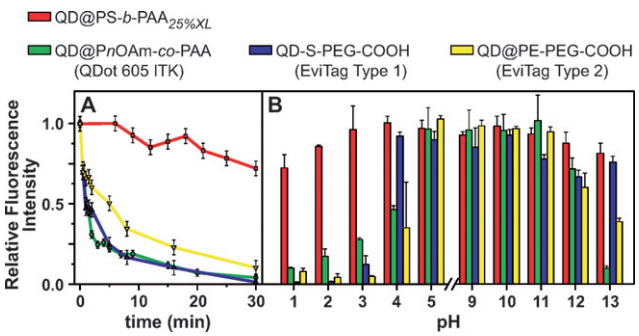


Fig. 2 (A) Time-elapsed fluorescence intensity of four QDs incubated in H_2O at 65 °C. (B) Relative fluorescence intensity of four QDs after 1 h incubation in aqueous HCl (pH 1–5) or NaOH (pH 9–13).

and at acidic pH was significantly more stable than other QDs (Fig. 2). We also found QD@PS-*b*-PAA$_{XL}$ to be more stable in concentrated Lewis-basic buffers such as HEPES and Tris (see ESI‡). We propose that the improved stability of QD@PS-*b*-PAA$_{XL}$ is due to the glassy nature of the hydrophobic PS block, which both keeps the surfactant chains from kinetic dissociation from the particle surface and creates a thick hydrophobic barrier against interactions between the nanoparticles and their aqueous surroundings. TEM images of CdSe@ZnS@PS-*b*-PAA$_{XL}$ after exposure to these conditions illustrated that the structures of the polymer surfactant shells were not changed, while other particles were either aggregated or etched under the same conditions (see ESI‡). In principle, this stability could be important for application of QDs as fluorescent tags in high-temperature biotechnological protocols such as the polymerase chain reaction (PCR).

The use of QDs for in vivo imaging requires that the particles also be mechanically stable to the stress of interacting with cell and tissue surfaces. Even though there is no covalent bond between the copolymer amphiphile and the encapsulated QD in QD@PS-*b*-PAA$_{XL}$, we found that the integrity of the copolymer coating—as determined by sustained fluorescence in vivo—was just as stable as that of covalently tethered polymer. Aqueous suspensions of CdSe@ZnS@PS-*b*-PAA$_{XL}$, CdSe@ZnS-S-PEG-COOH, and CdSe@ZnS@PE-PEG-COOH were injected into the common cardinal veins of zebrafish embryos.[21,22] All of these carboxylate-functionalized QDs adhered nonspecifically to the vascular endothelium within minutes of injection, as indicated by bright, localized spots in the fluorescence images. Previous work has shown that methoxy- or hydroxy-terminated poly(ethylene glycol) coatings can reduce nonspecific adsorption of QDs to epithelial cells.[23] However, CdSe@ZnS@PS-*b*-PAA$_{XL}$ particles in which unreacted carboxylates in the polymer shell were modified with α-methoxy-ω-amino-PEG (MW 2000 g/mol) *via* EDC coupling still adhered nonspecifically to the vasculature of zebrafish when injected. Welch, Wooley and coworkers have previously observed that PEGylation of shell-crosslinked PS-*b*-PAA block copolymer micelles had a complex effect on their biodistribution in Sprague–Dawley rats, decreasing nonspecific uptake from blood in lungs but not affecting uptake in liver, spleen or kidney.[24] (Vasculature was not separately analyzed in these experiments.) So, if CdSe@ZnS@PS-*b*-PAA$_{XL}$ particles behave similarly to empty PS-*b*-PAA$_{XL}$ micelles, it is not clear whether PEGylation of CdSe@ZnS@PS-*b*-PAA$_{XL}$ should have diminished nonspecific vascular adsorption or not.

In the case of CdSe@ZnS@PS-*b*-PAA$_{XL}$ (Fig. 3) and CdSe@ZnS-S-PEG-COOH (data not shown), the particles remained clearly fluorescent for the duration of observation (2 days). By contrast, CdSe@ZnS@PE-PEG-COOH lost fluorescence within minutes of injection (see ESI‡). Previous studies of quantum dots in circulation have shown that other covalent and polymer coatings also stabilize the fluorescence of CdSe@ZnS in vivo.[25–27] In all of these cases, the specific physical interaction between the nanoparticle and coating is critical, and we argue that the stability of CdSe@ZnS@PS-*b*-PAA$_{XL}$ in vivo is due to the physical integrity of the glassy, block-copolymer amphiphile shell under the shear mixing and stress of blood flow.

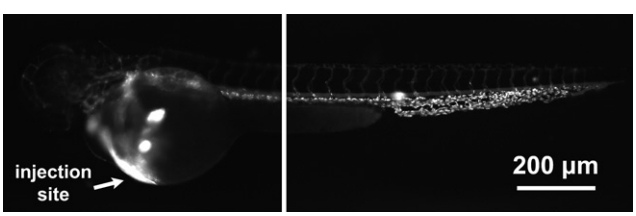


Fig. 3 Two (composited) epifluorescence images of a zebrafish embryo, 5 min after injection with QD@PS-*b*-PAA$_{XL}$. Images were obtained with an ET-DSRed filter set (Chroma Technology, Rockingham, VT).

In summary, crosslinked, glassy, block copolymer surfactants enhance the stability of QDs against extreme environments, and may contribute to the use of QDs in biological or biotechnological protocols requiring these conditions. There are certainly other problems with some biological applications of QDs that are not addressed by the crosslinked surfactant coatings described here. For example, although the 25–50 nm diameters of QD@PS-*b*-PAA$_{XL}$ particles are in the appropriate range for endocytosis,[28,29] much smaller QDs are required for monovalent labeling of single cell-surface receptors.[30,31] For the protocols involving high temperature or shear—such as PCR or vascular staining—QD@PS-*b*-PAA$_{XL}$ particles may offer unique advantages.

Acknowledgements

This work was supported by the U.S. National Institutes of Health (EB003809) and the State of Minnesota.

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